PHENCYCLIDINE (PCP), IN NANOMOLAR CONCENTRATIONS, BINDS TO SYNAPTOSOMES AND BLOCKS CERTAIN POTASSIUM CHANNELS

Covalent Labeling of K Channels with PCP

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Phencyclidine (1-[1-phenyclcyclohexyl] piperidine; "PCP," or "angel dust") is a drug of abuse that produces a schizophrenia-like behavioral syndrome in man (1). The behavioral effects have been attributed to PCP's enhancement of release of multiple neurotransmitters (1); this may be the consequence of potassium (K) channel block at central nervous system (CNS) nerve terminals (2).

RESULTS AND DISCUSSION

To study K conductance in presynaptic terminals we measured rubidium efflux from ^{86}Rb-loaded, pinched-off rat brain presynaptic nerve terminals (synaptosomes) that were prepared as described elsewhere (3). Synaptosomes retain considerable functional integrity; for example, they accumulate K (or Rb), and have membrane potentials that normally approximate the K equilibrium potential (4). When depolarized, for example by raising the external K concentration ($[K]_{out}$), ^{86}Rb efflux from the ^{86}Rb-loaded terminals is increased. The increment in ^{86}Rb efflux is graded with $[K]_{out}$. This K-stimulated ^{86}Rb efflux is probably mediated by K channels because Rb is known to pass through K-selective channels nearly as well as K itself (5), and because this efflux is blocked by a number of "typical" K channel blockers, including tetraalkylammonium ions, aminopyridines, quinine sulfate and barium (6). Thus, the K-stimulated ^{86}Rb efflux appears to be a useful measure of K conductance in the synaptosomes, which are too small to impale with microelectrodes. From the pharmacology of the K-stimulated ^{86}Rb efflux (e.g., see below), as well as its dependence, in part, on external Ca, we conclude that there are several types of K channels present in the heterogeneous population of nerve terminals.

As illustrated in Fig. 1, PCP blocks the K-stimulated ^{86}Rb efflux from synaptosomes. Particularly noteworthy is the fact that the dose-response curve (Fig. 1) is biphasic: ~20% of the stimulated efflux is blocked by 10–50 nM PCP, whereas concentrations >10 μM are required to inhibit the efflux further. A similar biphasic inhibition was observed with a behaviorally active analogue, *m*–NH_3–PCP (2). In contrast, two behaviorally inactive analogues, *m*–NO_2–PCP and PCC (1-piperidinocyclohexane carbonitrile) blocked the ^{86}Rb efflux only slightly, at high concentrations (>10 μM), and did not show a biphasic effect (2, 7). This correlation between the high-affinity block of ^{86}Rb efflux and the behavioral activity raises the possibility that the two phenomena are causally related.

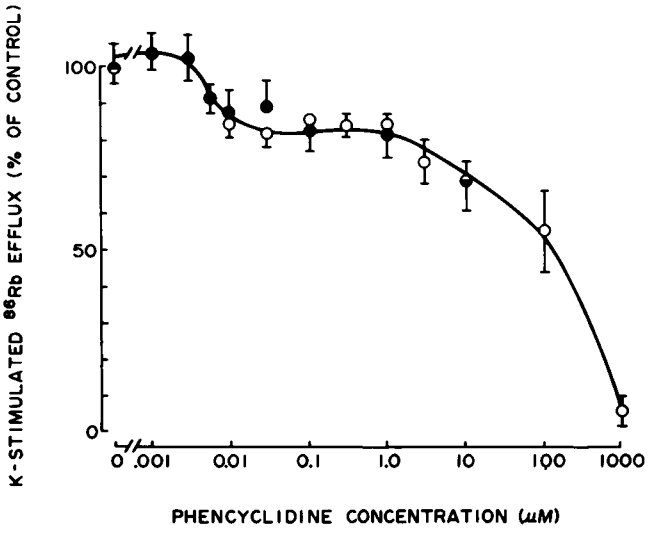

FIGURE 1 Dose-response curves illustrating the effects of PCP on K-stimulated ^{86}Rb efflux from synaptosomes. The data are from two different synaptosome preparations (○ and ●). Up to 1 mM PCP had no measureable effect on the amount of ^{86}Rb retained when the synaptosomes were incubated in 5 mM K (*5K*) solution (not shown; see reference 2). However, the ^{86}Rb-loaded synaptosomes that were diluted into the 75 mM K (*75K*) efflux solution without PCP, and incubated for 20 s, retained only ~70% as much ^{86}Rb as those diluted into 5K solution. The difference between the ^{86}Rb retained after incubation in 5-K and 75-K solutions corresponds to the "K-stimulated ^{86}Rb efflux." This difference is graphed in the figure; the difference observed in the absence of PCP corresponds to 100% on the graph. As illustrated, raising the PCP concentration in the efflux solution reduced the loss of ^{86}Rb from the synaptosomes incubated in the 75-K solution. Each data point corresponds to the difference between the means of determinations each in 5-K and 75-K solutions; bars indicate ± SEM (from reference 7 with permission).

We also measured the binding of ^{3}H-PCP to synaptosomes under conditions similar to those employed for the ^{86}Rb efflux experiments. As illustrated in Fig. 2, the ^{3}H–PCP binding curve in K-media is also biphasic. Scatchard analysis of the data from five such experiments indicates that there are ~0.5 pmol/mg synaptosome protein of high-affinity PCP binding sites, with an apparent dissociation constant, K_D, of ~60 nM; there are ~240 pmol/mg protein of low-affinity sites, with a K_D of ~115 μM. If the high-affinity sites are distributed uniformly on the terminals, 0.5 pmol/mg protein corresponds to ~20 sites per terminal. The similarity between the biphasic curves in Figs. 1 and 2 are consistent with the idea that the high-affinity PCP binding sites correspond to the K channels that are blocked by the nanomolar concentrations of PCP.

When the ^{3}H-PCP binding studies are carried out in nondepolarizing media with 5 mM K, rather than in K-rich depolarizing media, there is no high-affinity binding (Fig. 2, solid symbols). This implies that the PCP binds with high affinity only to the open K channels; these data are consistent with the observation that PCP block of K channels is use-dependent (2).

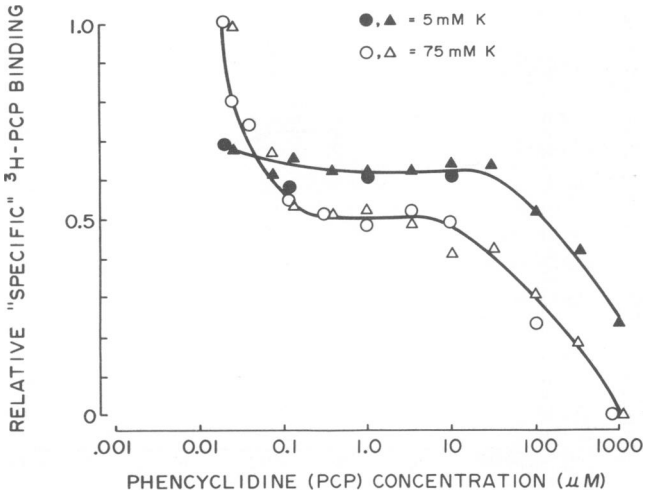

FIGURE 2 ^{3}H-PCP binding to synaptosomes: effect of external K concentration. A low concentration of high specific activity ^{3}H–PCP was added to all samples, along with varying amounts of unlabeled PCP to give the final concentrations indicated on the abscissa. The ^{3}H–PCP counts in the tissue blanks (no synaptosomes) at each PCP concentration (~10% of the counts obtained in the presence of synaptosomes) were subtracted from the respective synaptosome samples. The net counts retained on the filters after incubation of the synaptosomes with 1 mM unlabeled PCP in 75 K medium (~2,000 CPM in these experiments) were then subtracted from all other samples to obtain the "specific" ^{3}H–PCP binding. As shown in the graph, these data were then scaled to a value of 1.0 for the binding (^{3}H–PCP counts) in the 75-K incubation solution at the highest PCP-specific activity (= lowest PCP concentrations). The data are from two experiments (circles and triangles). On an absolute scale, 1.0 corresponds to ~6,000 CPM/0.64 mg protein (the amount per filter), and O, corresponds to ~2,000 CPM/0.64 mg protein (with 1 mM unlabeled PCP present). The incubation media contained 20 nM ^{3}H–PCP (48 Ci/nmol), plus unlabeled PCP as indicated. (From reference 7, with permission; *q. v.* for details).

PCP is itself photoactivatable, and can be linked covalently to certain membrane proteins (8). When synaptosomes are suspended in media containing ^{3}H–PCP and irradiated with 254 nm UV light, some of the ^{3}H becomes covalently bound to the synaptic plasma membranes (7): it can no longer be displaced by excess unlabeled PCP, or by extensive washing, osmotic shock, and sucrose gradient centrifugation. If varying amounts of unlabeled PCP are present in the K-rich incubation media at the time of UV irradiation, the concentration dependence curve obtained for covalently bound ^{3}H–PCP is virtually identical to the biphasic curve in Fig. 2 (see reference 7). These findings indicate that the high-affinity PCP binding sites on the plasma membrane can be covalently labeled. Preliminary data from Na dodecylsulfate-polyacrylamide gel electrophoresis and radiofluorographic analysis of the gels suggest that ^{3}H–PCP binds, with high affinity, to a 220,000 molecular weight polypeptide; this may be associated with (or part of) the K channels that are blocked by the low concentrations of PCP (Fig. 1).

Our data show that the behavioral potency of PCP and its analogues can be correlated with their ability to block, with high affinity, certain K channels in presynaptic terminals. This suggests that these K channels may play a critical role in the control of behavior at the cellular level. Blockage of these channels would be expected to prolong the action potentials that invade the terminals, thereby increasing Ca entry and evoked transmitter release. This alteration of synaptic transfer could, perhaps, even produce long-term changes in CNS function. Moreover, because of the resemblance between the behavioral defects of PCP-intoxicated individuals and some patients with primary schizophrenia, these data raise the possibility that some schizophrenics may have defective ion channels in their CNS neurons.

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SOLVENT-SOLUTE INTERACTIONS WITHIN THE NEXAL MEMBRANE

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The nexus, or gap junction, is thought to contain aqueous intercellular channels with diameters of ~1.5 nm (2, 13). Both physiological (20, 21) and anatomical data (14) have contributed to the aqueous channel concept. More recent findings in the septate giant axon system (7) have shown that the temperature dependence of junctional permeability to dichlorofluorescein decreases sharply (blocks) at 4° ± 0.5°C in H_2O and 6°C in D_2O. Action potential propagation still continues across the junction well below 4°C in either solvent. The block temperature can be defined as that temperature at which no appreciable dye diffusion can be observed across the junction for one to two hours. In reality the permeability of the junction has fallen below the ability of the photomultiplier system to perceive the movement of dye across the septa over the time interval indicated. Although the diffusion rates were unmeasurable at block temperature, some dye transfer was observable with long time intervals (14–20 h). The junctions studied were nexuses contained within the septa of the septate median giant axon of earthworm (5, 8, 10).

To elucidate further the nature of ion and anion movement within the junction, the involvement of solvent-solute, solute-channel and solvent-channel interactions was investigated for a number of fluorescent probes in both deuterium oxide (D_2O) and water (H_2O). The probes used were carboxyfluorescein, Lucifer Yellow CH, Lucifer Yellow 37 and diiodofluorescein (Fig. 2). In addition, junctional membrane conductance was monitored in D_2O and H_2O (4). Temperature regulation was accomplished by use of a Cambion temperature control device. The pH and pD were adjusted such that both equaled 7.3 (19).

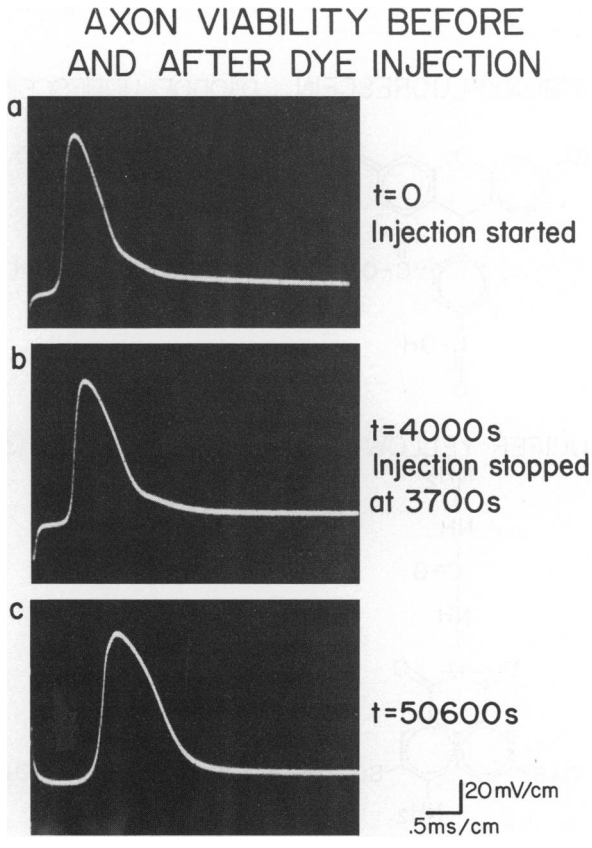

FIGURE 1 A microelectrode filled with dye (Lucifer Yellow CH) was inserted into an axon in H_2O saline. *a*, an action potential is shown. Stimulating electrodes were 1 cm away from the site of recording. *b*, the same cell 4,000 s later after a 3,700 s injection. The microelectrode was removed. The cell was scanned and diffusion monitored and *c* at 50,600 s a 3 M KCL microelectrode was inserted into the same cell and the nerve cord was once again stimulated. The threshold was larger and the cell was partially depolarized (≈10 mV).

RESULTS AND DISCUSSION

The fluorescent probes were inserted by iontophoresis into the septate axon system. Hyperpolarizing current pulses 50